DOCKET NO.: TIBO-0011 **Application No.:** 09/599,877

Office Action Dated: August 26, 2004

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

- 1. (Currently amended) A method for determining the level of resistance of HIV resensitization of HIV-1 to an HIV-RT inhibitorAZT, comprising:
- a) providing a reaction well with the reaction products of substances following reaction components comprising:
 - i) at least one template for an [[HIV]]HIV-1 RT enzyme[[,]];
 - ii) at least one primer[[,]];
 - <u>iii)</u> at least one detectable dNTP substrate[[,]];
 - iv) at least one HIV RT inhibitor AZT[[,]]; and
- v.) at least one ribonucleotide chosen from ATP or GTP, or at least one pyrophosphate;
- b) performing an enzymatic kinetics assay that permits the measurement of multiple chain termination events by adding to the reaction well an [[HIV]]HIV-1 RT enzyme chosen from a wild-type RT enzyme, and a mutant selected from the group consisting of M41L/T215Y; M41L/M184V/T215Y; M41L/D67N/K70R/T215Y; M41L/D67N/K70R/T215Y; M41L/D67N/K70R/M184V/L210W/R211K/L214F/T215Y; T69S-SS; T69S-SG; T69S-AG; and T69S-SS/T215Y, wherein said numbering scheme is based upon the prototypical isolate HIV-1_{BH-10},

wherein said [[HIV]]<u>HIV-1</u> RT enzyme incorporates the at least one detectable dNTP substrate or at least one HIV RT inhibitor <u>AZT</u> into said template;

- c) determining RT activity by measuring the amount of the detectable dNTP substrate incorporated into the template;
- d) repeating steps b) and c) replacing the wild-type RT enzyme with a mutant RT enzyme; and
 - e) determining the level of resistance of HIV resensitization of HIV-1 to the HIV RT inhibitor AZT by comparing the RT activity of the wild-type RT enzyme with the RT activity of the mutant RT enzyme;
 - wherein the level of resistance of HIV resensitization of HIV-1 to an HIV RT inhibitor AZT is determined.

DOCKET NO.: TIBO-0011 PATENT

Application No.: 09/599,877

Office Action Dated: August 26, 2004

2. (Original) The method of claim 1, wherein the template is bound to the reaction well and is chosen from poly-rA or a heteropolymer RNA or DNA.

- 3. (Original) The method of claim 1, wherein the primer is chosen from oligo-dt or a primer that is complementary to the heteropolymer template.
- 4. (Original) The method of claim 1, wherein the detectable dNTP substrate is chosen from a radioactive labeled dNTP.
- 5. (Original) The method of claim 1, wherein the detectable dNTP substrate is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
- 6. (Original) The method of claim 1, wherein the detectable dNTP substrate binds to an optical tracer or a radioactive labeled tracer.
- 7. (Original) The method of claim 6, wherein the optical tracer is capable of being detected by fluorescence, luminescence, or absorption spectrometry.
- 8. (Original) The method of claim 6, wherein the detectable dNTP precursor is bromo-deoxyuridine-triphosphate.
- 9. (Original) The method of claim 7, wherein the optical tracer is a monoclonal anti-BrdU antibody, conjugated to alkaline phosphatase.
- 10. (Currently amended) The method of claim 1, wherein the [[HIV]]<u>HIV-1</u> RT inhibitor is chosen from AZT, 3TG, ddI, ddC, d4T, and abacavir.
- 11. (Currently amended) The method of claim 1, wherein the [[HIV]]<u>HIV-1</u> RT inhibitor is chosen from a nucleoside or a nucleoside analog.
- 12. (Currently amended) The method of claim 11, wherein the [[HIV]]<u>HIV-1</u> RT inhibitor is a triphosphate form of the [[HIV]]<u>HIV-1</u> RT inhibitor.
- 13. (Cancelled)

DOCKET NO.: TIBO-0011 PATENT

Application No.: 09/599,877

Office Action Dated: August 26, 2004

14. (Currently amended) The method of claim 1, wherein the HIV-1 mutant RT enzyme contains an insertional mutation at nucleotide triplet encoding codon 69 amino acid insertion between codons 69 and 70.

Claims 15-19 cancelled

- 20. (Currently amended) A method for determining the effect of at least one mutation in an [[HIV]]<u>HIV-1</u> RT enzyme on the resistance of HIV resensitization of HIV-1 to an HIV-RT inhibitorAZT, comprising:
- a) providing a reaction well with the reaction products of substances following reaction components comprising:
 - i)_at least one template for an [[HIV]]HIV-1 RT enzyme[[,]];
 - ii) at least one primer[[,]];
 - iii) at least one detectable dNTP substrate[[,]];
 - iv) at least one HIV RT inhibitor AZT[[,]]; and
- <u>v.)</u> at least one ribonucleotide chosen from ATP or GTP, or at least one pyrophosphate;
- b) performing an enzymatic kinetics assay that permits the measurement of multiple chain termination events by adding to the reaction well an HIV RT enzyme, and a mutant selected from the group consisting of M41L/T215Y; M41L/M184V/T215Y; M41L/D67N/K70R/T215Y; M41L/D67N/K70R/M184V/T215Y; M41L/D67N/K70R/M184V/L210W/R211K/L214F/T215Y; T69S-SS; T69S-SG; T69S-AG; and T69S-SS/T215Y, wherein said numbering scheme is based upon the prototypical isolate HIV-1_{BH-10},

wherein said [[HIV]]<u>HIV-1</u> RT enzyme incorporates the at least one detectable dNTP substrate or the at least one [[HIV]]<u>HIV-1</u> RT inhibitor into said template;

- c) determining RT activity by measuring the amount of the detectable dNTP substrate incorporated into the template;
- d) repeating steps a) through c) in a new reaction well wherein the [[HIV]]HIV-1 RT enzyme of step b) is chosen from at least one mutant RT enzyme;
 - e) comparing the RT activity in the different reaction wells; and

DOCKET NO.: TIBO-0011 **Application No.:** 09/599,877

Office Action Dated: August 26, 2004

f) determining the effect of the at least one mutation on the resistance of [[HIV]]<u>HIV-1</u> to an HIV RT inhibitor AZT using step e);

wherein the effect of at least one mutation in an [[HIV]]<u>HIV-1</u> RT enzyme on the resistance of HIV resensitization of HIV-1 to an HIV RT inhibitorAZT can be determined.

- 21. (Currently amended) A method for rapid screening the effects of mutations on HIV resistance resensitization of HIV-1 to an HIV RT inhibitor AZT, comprising:
- a) providing an array of reaction wells, each reaction well with the reaction products of substances following reaction components comprising:
 - i) at least one template for an [[HIV]]HIV-1 RT enzyme[[,]];
 - ii) at least one primer[[,]];
 - iii) at least one detectable dNTP substrate[[,]];
 - iv) at least one HIV RT inhibitor AZT[[,]]; and
- v.) at least one ribonucleotide chosen from ATP or GTP, or at least one pyrophosphate;
- b) performing an enzymatic kinetics assay that permits the measurement of multiple chain termination events by adding to each reaction well a different [[HIV]]HIV-1 RT enzyme chosen from a wild-type RT enzyme or a mutant RT enzyme, and a mutant selected from the group consisting of M41L/T215Y; M41L/M184V/T215Y; M41L/D67N/K70R/M184V/T215Y; M41L/D67N/K70R/M184V/T215Y; M41L/D67N/K70R/M184V/L210W/R211K/L214F/T215Y; T69S-SS; T69S-SG; T69S-AG; and T69S-SS/T215Y, wherein said numbering scheme is based upon the prototypical isolate HIV-1_{BH-10},

wherein said [[HIV]]<u>HIV-1</u> RT enzyme incorporates the at least one detectable dNTP substrate or the at least one <u>HIV RT inhibitorAZT</u> into said template and wherein at least one wild-type RT enzyme is added to at least one reaction well;

- c) determining RT activity in each reaction well by measuring the amount of the detectable dNTP substrate incorporated into the template; and
- d) determining the effect of mutations on HIV resistance resensitization of HIV-1 of the HIV RT inhibitor AZT by comparing the RT activity of at least one wild-type RT enzyme with the RT activity of at least one mutant RT enzyme;

DOCKET NO.: TIBO-0011 PATENT

Application No.: 09/599,877

Office Action Dated: August 26, 2004

wherein the rapid screening the effects of mutations on HTV resistance resensitization of HIV-1 to an HIV-RT inhibitor AZT is determined.